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Kyle J. Waite

Z. Elizabeth Floyd

Patricia Arbour-Reily

Jacqueline M. Stephens
Louisiana State University

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Interferon-γ-induced Regulation of Peroxisome Proliferator-activated Receptor γ and STATs in Adipocytes*

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Kyle J. Waite, Z. Elizabeth Floyd, Patricia Arbour-Reily, and Jacqueline M. Stephens‡

From the Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

Interferon-γ (IFN-γ) is known primarily for its roles in immunological responses but also has been shown to affect fat metabolism and adipocyte gene expression. To further investigate the effects of IFN-γ on fat cells, we examined the effects of this cytokine on the expression of adipocyte transcription factors in 3T3-L1 adipocytes. Although IFN-γ regulated the expression of several adipocyte transcription factors, IFN-γ treatment resulted in a rapid reduction of both peroxisome proliferator-activated receptor (PPAR) protein and mRNA. A 48-h exposure to IFN-γ also resulted in a decrease of both CCAAT/enhancer-binding α and sterol regulatory element binding protein (SREBP-1) expression. The short half-life of both the PPARγ mRNA and protein likely contributed to the rapid decline of both cytosolic and nuclear PPARγ in the presence of IFN-γ. Our studies clearly demonstrated that the IFN-γ-induced loss of PPARγ protein is partially inhibited in the presence of two distinct proteasome inhibitors. Moreover, IFN-γ also inhibited the transcription of PPARγ, which was accompanied by a decrease in PPARγ mRNA accumulation. In addition, exposure to IFN-γ resulted in a substantial increase in STAT 1 expression and a small increase in STAT 3 expression. IFN-γ treatment of 3T3-L1 adipocytes (48–96 h) resulted in a substantial inhibition of insulin-sensitive glucose uptake. These data clearly demonstrate that IFN-γ treatment results in the development of insulin resistance, which is accompanied by the regulation of various adipocyte transcription factors, in particular the synthesis and degradation of PPARγ.

The adipocyte plays an active role in a variety of physiological and pathological processes regulating energy metabolism. The recent consideration of adipose tissue as an endocrine organ that secretes a variety of unrelated bioactive molecules has broadened our understanding of adipocyte function to exceed its previously considered passive role in lipid metabolism. A number of cell lines are available for studying adipocytes. The 3T3-L1 cell line differentiates under the controlled conditions of cell culture from fibroblasts, or preadipocytes, to cells with the morphological and biochemical properties of adipocytes (1, 2). The 3T3-L1 adipocytes are comparable with native adipocytes as they have the ability to accumulate lipid, respond to insulin, and secrete leptin. The major transcription factors involved in adipocyte gene regulation include peroxisome proliferator-activated receptor γ, proteins belonging to the CCAAT/enhancer-binding protein family, and adipocyte determination and differentiation-dependent factor 1, also known as sterol regulatory element-binding protein (reviewed in Refs. 3 and 4).

Recent studies have also suggested that the signal transducer and activator of transcription (STAT)[1] family of transcription factors may also be important in fat cells. The STAT family of transcription factors is comprised of seven family members (STATs 1, 2, 3, 4, 5A, 5B, and 6) that, in response to the stimulation of various receptors, mainly those for cytokines, are phosphorylated on tyrosine residues, which causes their translocation to the nucleus. Each STAT family member shows a distinct pattern of activation by cytokines and upon nuclear translocation can regulate the transcription of particular genes in a cell- or tissue-specific manner (5). In fat cells, the expression of STATs 1, 5A, and 5B is highly induced during differentiation and correlates with lipid accumulation (6, 7). The regulation of STAT expression has also been investigated in NIH 3T3 cells ectopically overexpressing C/EBPs β and δ, a condition that results in adipogenesis (8). In these studies, the expression of STATs 1, 5A, and 5B was induced in a PPARγ ligand-dependent fashion during adipogenesis (9). STATs 3 and 6 are also expressed in adipocytes, but the expression of these proteins does not change during differentiation. However, the tyrosine phosphorylation of STAT 3 occurs following the induction of differentiation, and studies with antisense STAT 3 suggest that this protein may be important in adipogenesis (10). Although the functions of STATs in fat cells have not been identified, numerous studies suggest that these transcription factors may be important regulators of adipocyte gene expression.

Interferon-γ (IFN-γ) is primarily known for its roles in immunological responses but also has been shown to affect fat metabolism and adipocyte gene expression. In adipocytes, IFN-γ treatment results in a decrease of lipoprotein lipase activity and increased lipolysis (11). In 3T3-F442 adipocytes, exposure to IFN-γ results in a decreased expression of lipoprotein lipase and fatty acid synthase. Also in various rodent preadipocyte cell lines, IFN-γ inhibits the differentiation of preadipocytes (12–14). We have recently shown that acute IFN-γ treatment of cultured and native adipocytes results in a

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‡ To whom correspondence should be addressed: Dept. of Biological Sciences, Louisiana State University, 508 Life Sciences Bldg., Baton Rouge, LA 70803; Tel.: 225-388-1749; Fax: 225-388-2597; E-mail: jsteph1@unix1.sncc.lsu.edu.

[1] The abbreviations used are: STAT, signal transducer and activator of transcription; C/EBP, CCAAT/enhancer-binding protein; ERK, extracellular signal-regulated kinase; TZD, thiazolidinedione; DMEM, Dulbecco's modified Eagle's medium; ENG, englitazone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulatory element-binding protein.

dose- and time-dependent activation of STATs 1 and 3 (15). Exposure of adipocytes to IFN-γ results in the tyrosine phosphorylation and nuclear translocation of STATs 1 and 3 in fat cells. Because IFN-γ has effects on adipocyte gene expression, we examined the effects of this cytokine on the expression of a variety of adipocyte transcription factors.

Although we observed that IFN-γ affected the expression of a number of adipocyte transcription factors, the most profound effect of IFN-γ was on the expression of PPARγ. PPARγ is a member of the nuclear hormone superfamily and exists as two isoforms, PPARγ1 and PPARγ2, which are transcribed from the same gene by the use of alternative promoters (16). PPARγ2 is 30 amino acids longer than PPARγ1 and is largely adipocyte-specific. Although expressed in a variety of other tissues, PPARγ1 is also predominately expressed in fat (17). Thiazolidinediones (TZDs) are high affinity synthetic ligands of PPARγ and have recently been shown to affect the degradation of this transcription factor (18). Our studies with IFN-γ also indicate that PPARγ is targeted to the proteasome for degradation, but this is not the only mechanism for the substantial effect that IFN-γ has on PPARγ expression. Our findings indicate that a newly identified inhibitor of PPARγ expression, IFN-γ, results in a substantial loss of PPARγ expression by regulating two cellular events as follows: 1) targeting PPARγ to the proteasome for degradation and 2) inhibiting the synthesis of PPARγ. Prolonged IFN-γ treatment of 3T3-L1 adipocytes also results in the development of insulin resistance and regulation of other adipocyte transcription factors and supports the hypothesis that PPARγ is involved in conferring insulin sensitivity.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco's modified Eagle's medium (DMEM) was purchased from Life Technologies, Inc. Bovine and fetal bovine serum was obtained from Sigma and Life Technologies, Inc., respectively. Murine interferon-γ (IFN-γ) was purchased from Roche Molecular Biochemicals. Actinomycin D was purchased from Calbiochem. Cycloheximide was purchased from Sigma. The nonphospho STAT antibodies were either monoclonal IgGs purchased from Transduction Laboratories or polyclonal IgGs from Santa Cruz Biotechnology Inc. A highly phosphospecific polyclonal antibody for STAT 1 (Y^{701}) was provided by Quality Controlled Biochemicals. PPARγ was a mouse monoclonal antibody from Santa Cruz Biotechnology Inc. SREBP-1, C/EBPα, and ERK1/ERK2 were rabbit polyclonal antibodies from Santa Cruz Biotechnology Inc.

Cell Culture—Murine 3T3-L1 preadipocytes were plated and grown to 2 days postconfluence in DMEM with 10% bovine serum. Medium was changed every 48 h. Cells were induced to differentiate by changing the medium to DMEM containing 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 1.7 μM insulin. After 48 h this medium was replaced with DMEM supplemented with 10% fetal bovine serum, and cells were maintained in this medium until utilized for experimentation.

Preparation of Whole Cell Extracts—Monolayers of 3T3-L1 adipocytes were rinsed with phosphate-buffered saline and then harvested in a nondenaturing buffer containing 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 0.5% Nonidet P-40, 1 μM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 50 trypsin inhibitory milliunits of aprotinin, 10 μM leupeptin, and 2 mM sodium vanadate. Samples were extracted for 30 min on ice and centrifuged at 15,000 rpm at 4 °C for 15 min. Supernatants containing whole cell extracts were analyzed for protein content using a BCA kit (Pierce) according to the manufacturer's instructions.

Preparation of Nuclear/Cytosolic Extracts—Cell monolayers were rinsed with phosphate-buffered saline and then harvested in a nuclear homogenization buffer (NHB) containing 20 mM Tris (pH 7.4), 10 mM NaCl, and 3 mM $MgCl_2$. Nonidet P-40 was added to a final concentration of 0.15%, and cells were homogenized with 16 strokes in a Dounce homogenizer. The homogenates were centrifuged at 1500 rpm for 5 min. Supernatants were saved as cytosolic extract, and the nuclear pellets were resuspended in 0.5 volume of NHB and centrifuged as before. The pellet of intact nuclei was resuspended again in 0.5 of the original volume of NHB and centrifuged again. A small portion of the nuclei was used for trypan blue staining to examine the integrity of the nuclei. The majority of the pellet (intact nuclei) was resuspended in an extraction buffer containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 25% glycerol. Nuclei were extracted for 30 min on ice and then placed at room temperature for 10 min. Two hundred units of DNase I were added to each sample, and tubes were inverted and incubated an additional 10 min at room temperature. Finally, the sample was subjected to centrifugation at 15,000 rpm at 4 °C for 30 min. Supernatants containing nuclear extracts were analyzed for protein content.

Gel Electrophoresis and Immunoblotting—Proteins were separated in 5, 7.5, or 12% polyacrylamide (acrylamide from National Diagnostics) gels containing sodium dodecyl sulfate (SDS) according to Laemmli (19) and transferred to nitrocellulose (Bio-Rad) in 25 mM Tris, 192 mM glycine, and 20% methanol. Following the transfer, the membrane was blocked in 4% milk for 1 h at room temperature. Results were visualized with horseradish peroxidase-conjugated secondary antibodies (Sigma) and enhanced chemiluminescence (Pierce).

RNA Analysis—Total RNA was isolated from cell monolayers with TriZOL (Life Technologies, Inc.) according to the manufacturer's instruction with minor modifications. For Northern blot analysis, 20 μg of total RNA was denatured in formamide and electrophoresed through a formaldehyde-agarose gel. The RNA was transferred to Zeta Probe-GT (Bio-Rad), cross-linked, hybridized, and washed as previously described (20). Probes were labeled by random priming using the Klenow fragment (Promega) and [α-^{32}P]dATP (PerkinElmer Life Sciences).

Determination of 2-Deoxyglucose—The assay of 2-[^{3}H]deoxyglucose was performed as previously described (21). Prior to the assay, fully differentiated 3T3-L1 adipocytes were serum-deprived for 2–4 h. Uptake measurements were performed in triplicate under conditions where hexose uptake was linear, and the results were corrected for nonspecific uptake and absorption determined by 2-[^{3}H]deoxyglucose uptake in the presence of 5 μM cytochalasin B (Sigma). Nonspecific uptake and absorption were always less than 10% of the total uptake.

Nuclei Isolation and Run-on Transcription Assays—Following exposure of fully differentiated adipocytes to IFN-γ for 1 h, the cell monolayers (six 10-cm plates per time point) were washed once with ice-cold phosphate-buffered saline and nuclei were isolated, and run-on transcription assays were performed as we have previously described (20).

RESULTS

The expression of adipocyte transcription factors was examined following a time course of IFN-γ treatment on fully differentiated 3T3-L1 adipocytes. As shown in Fig. 1, immunoblotting of whole cell extracts demonstrated that IFN-γ treatment resulted in a significant decrease in PPARγ2 (*upper band*) and -γ1(*lower band*) within 24 h and resulted in a notable decline in C/EBPα. The expression of STATs 1 and 3 increased following a 24-h IFN-γ treatment. The expression of STATs 5A, 5B, and 6 was not regulated by exposure to IFN-γ treatment. Also, the expression of SREBP-1 decreased after a 48-h treatment. The spliced 67-kDa form of SREBP-1 was similarly decreased with IFN-γ treatment (data not shown).

As shown in Fig. 1, a 24-h treatment of IFN-γ resulted in a substantial loss of PPARγ2 and -γ1 protein expression. Therefore, we examined the effects of IFN-γ over a 24-h time course. Whole cell extracts were isolated from fully differentiated 3T3-L1 adipocytes that were treated with IFN-γ for the various times indicated in Fig. 2. Interestingly, IFN-γ resulted in a substantial loss of PPARγ2 and -γ1 expression within 6 h. In addition, we observed a striking increase in STAT 1 expression between 8 and 12 h and a small increase in STAT 3 during this time period. There was no change in STAT 5A during this time course. JAK 1, the kinase that activates STAT 1 in adipocytes, increases slightly with IFN-γ treatment. In addition, fatty acid synthase expression decreased with IFN-γ treatment.

Clearly, an analysis of whole cell extracts reveals a substantial loss of PPARγ2 and -γ1 expression in adipocytes following IFN-γ treatment. However, it was unclear whether IFN-γ had any effect on the amount of PPARγ proteins present in the adipocyte nucleus. To further examine the inhibition of PPARγ by IFN-γ, we performed another time course in which adipo-

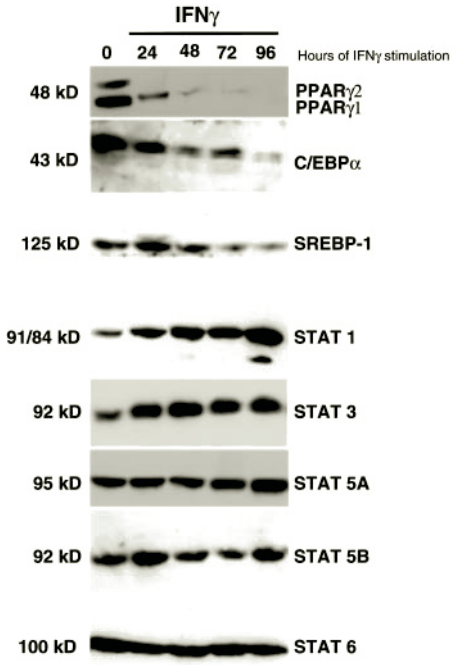

FIG. 1. **The effects of IFN-γ on the expression of adipocyte transcription factors.** Whole cell extracts were prepared from fully differentiated 3T3-L1 adipocytes following a treatment with 100 units/ml IFN-γ for 0, 24, 48, 72, or 96 h. Cells were treated every 24 h with a fresh bolus of IFN-γ. Extracts were prepared as described under "Experimental Procedures." One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. The molecular mass of each protein is indicated to the *left* of the blot in kilodaltons. The detection system was horseradish peroxidase-conjugated secondary antibodies (Sigma) and enhanced chemiluminescence (Pierce). This is a representative experiment independently performed three times.

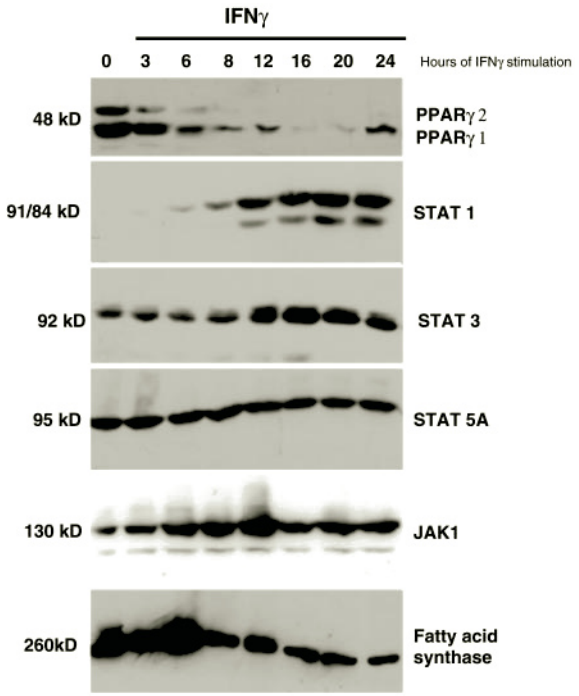

FIG. 2. **IFN-γ treatment results in a rapid loss of PPARγ expression in adipocytes.** Whole cell extracts were prepared from fully differentiated 3T3-L1 adipocytes following a treatment with 100 units/ml IFN-γ as indicated at the *top* of the figure. One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. The molecular mass of each protein is indicated to the *left* of the blot in kilodaltons. Samples were processed and results were visualized as described in the legend to Fig. 1. This is a representative experiment independently performed three times. (*JAK1*, the kinase that activates STAT1 in adipocytes.

cytes were fractionated to isolate cytosolic and nuclear extracts. As shown in the *top panel* of Fig. 3, the majority of PPARγ2 and -γ1 protein was present in the nucleus, and the amount of nuclear PPARγ protein was substantially reduced after 6 h. A darker exposure of this blot indicates the presence of PPARγ proteins in the cytosol in untreated adipocytes and cells that were exposed to IFN-γ for 30 min. However, following a 6-h or greater IFN-γ treatment, there was no detectable PPARγ2 or -γ1 in the cytosol and a significant loss of both PPARγ isoforms in the nucleus. We also observed an increase in STAT 1 in the cytosol between 6 and 12 h and the presence of activated STAT 1 in the nucleus following a 30-min treatment with IFN-γ. Detection of the phosphorylated form of STAT 1 was performed with an antibody specific for phosphorylation on tyrosine 701 (STAT 1 Y^{701}). Analysis with either one of these STAT 1 antibodies demonstrates the presence of STAT 1 in the nucleus following a 30-min IFN-γ stimulation. However, the STAT 1 Y^{701} antibody is more sensitive, and we observed this protein in the nucleus even after a 12-h IFN-γ treatment. We have previously reported that STAT 5A is present in the nucleus of adipocytes under basal conditions (15), and IFN-γ treatment does not cause a redistribution of this protein. Therefore, STAT 5A (Fig. 3, *bottom panel*) is shown to indicate the even loading of both cytosolic and nuclear samples.

The rapid loss of PPARγ1 and -γ2 proteins in the presence of IFN-γ suggested that the PPARγ proteins may be labile. Therefore, we examined the decay of PPARγ and STATs in 3T3-L1 adipocytes. Whole cell extracts were isolated from 3T3-L1 adipocytes at various times following the addition of 5 μM cycloheximide (+*CH*) or ethanol (−*CH*), a vehicle control. As shown in Fig. 4, the inhibition of protein synthesis resulted in the loss

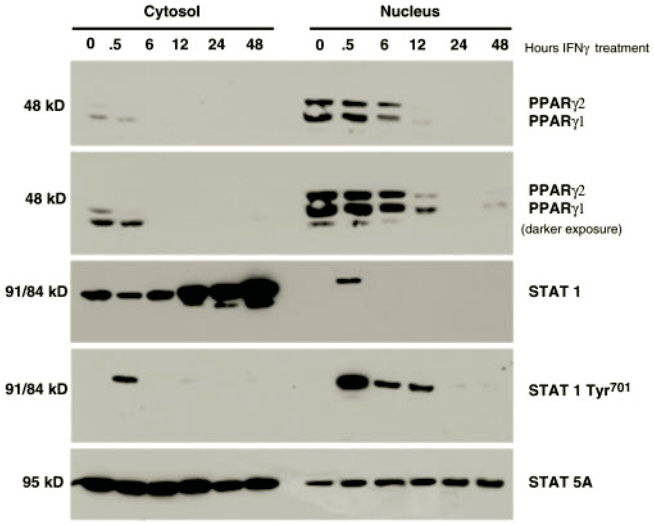

FIG. 3. **IFN-γ treatment results in a decrease of nuclear PPARγ and an increase in cytosolic STAT 1 in adipocytes.** Cytosolic and nuclear extracts were isolated from fully differentiated 3T3-L1 adipocytes following treatment with IFN-γ as indicated at the *top* of the figure. One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in the legend to Fig. 1. This is a representative experiment independently performed two times.

of PPARγ by 12 h with over half of the protein decayed by 6 h. A log plot of the remaining protein *versus* time was used to estimate the half-life of PPARγ and of adipocyte-expressed STAT proteins. The estimated half-life of these proteins is indicated in Fig. 4 and is an average calculation of three inde-

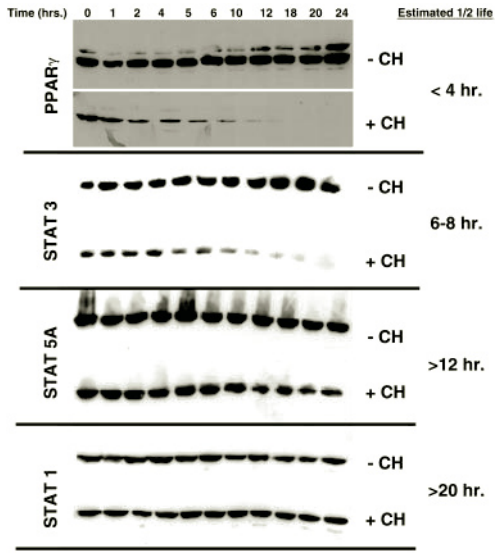

FIG. 4. **The turnover of PPARγ and adipocyte-expressed STAT proteins.** Whole cell extracts were prepared from 3T3-L1 adipocytes following various periods of treatment with 5 μM cycloheximide (+*CH*) or ethanol (−*CH*), a vehicle control. One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in the legend to Fig. 1. This is a representative experiment independently performed three times.

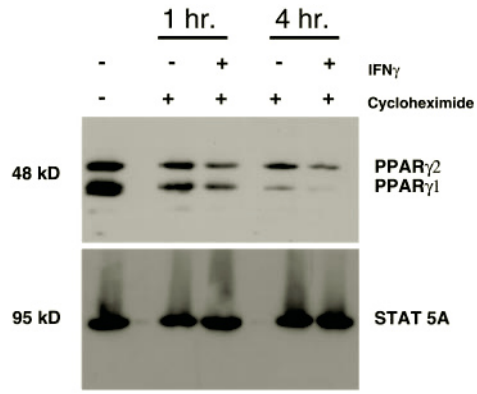

FIG. 5. **PPARγ turnover is increased in the presence of IFN-γ.** Whole cell extracts were prepared from 3T3-L1 adipocytes following various periods of treatment with 5 μM cycloheximide or ethanol in the presence or absence of IFN-γ. One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in the legend to Fig. 1. This is a representative experiment independently performed three times.

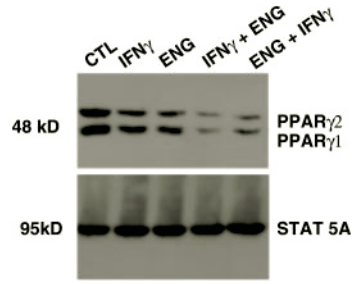

FIG. 6. **The IFN-γ-induced increase of PPARγ expression is even greater in the presence of englitazone.** Whole cell extracts were prepared from fully differentiated 3T3-L1 adipocytes following a 5-h treatment of IFN-γ, ENG, IFN-γ + ENG (added 1 h after the addition of IFN-γ), and ENG + IFN-γ (added 1 h after the addition of ENG). One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in the legend to Fig. 1. *CTL*, control.

pendent experiments. PPARγ1 and -γ2 are labile compared with the STAT proteins, which have half-lives at least twice as long as the PPARγ proteins. We also investigated the effect of IFN-γ on PPARγ in the presence of cycloheximide. Because of experimental variability, it was difficult to quantitate the decrease in the half-life of the PPARγ proteins in the presence of IFN-γ. However, in each experiment, the decay of the PPARγ was quicker in the presence of IFN-γ, as shown in Fig. 5. Adipocytes were treated with 5 μM cycloheximide in the presence or absence of IFN-γ, and whole cell extracts were isolated at 0, 1, and 4 h. As shown in Fig. 5, the decay of both PPARγ2 and -γ1 is increased in the presence of IFN-γ with a complete loss of PPARγ1 at 4 h.

TZD treatment has also been shown to decrease PPARγ expression. Therefore, we compared the effects of IFN-γ and englitazone (ENG), a TZD, on the expression of PPARγ in adipocytes. As shown in Fig. 6, fully differentiated adipocytes were exposed to IFN-γ or ENG alone or in combination. In the first combination, adipocytes were treated with IFN-γ 1 h prior to the addition of englitazone. In the second combination, adipocytes were treated with englitazone 1 h prior to the addition of IFN-γ. For each combination, whole cell extracts were isolated 5 h after initiation of the experiment. These results demonstrate that the combination of both inhibitors of PPARγ expression resulted in an even greater decrease in PPARγ expression than one agonist alone.

The results of the cycloheximide experiments in Fig. 5 suggest that the decay of PPARγ2 and -γ1 is increased in the presence of IFN-γ. Therefore, we examined PPARγ expression in the presence of proteasome inhibitors. As shown in Fig. 7, a 6-h treatment of either epoxomicin or lactacystin had little effect on the levels of PPARγ2 or -γ1 protein. A 6-h IFN-γ treatment resulted in a substantial loss of PPARγ protein, but the IFN-γ-induced loss of PPARγ2 and -γ1 was inhibited in the presence of either epoxomicin or lactacystin. Notably, the presence of these two different proteasome inhibitors did not restore PPARγ2 and -γ1 to the levels found in untreated adipocytes, suggesting that protein degradation is only one manner in which IFN-γ regulates PPARγ expression. In the presence of the two proteasome inhibitors, there were no differences in the levels of any STATs or ERK1/ERK2. Interestingly, the IFN-γ-induced increase in STAT 1 was blunted in the presence of epoxomicin or lactacystin, suggesting that the IFN-γ-induced increase in STAT 1 may be dependent on the degradation of some protein(s).

These experiments indicate that, in addition to having an effect on the turnover of the PPARγ proteins, there is presumably another means by which IFN-γ causes a decrease in PPARγ expression. Therefore, we examined the effect of IFN-γ on PPARγ mRNA accumulation. As shown in Fig. 8, a 2-h IFN-γ treatment resulted in a substantial loss of PPARγ mRNA. Northern blot analysis cannot distinguish between the two forms of PPARγ. A decrease in C/EBPα and *GLUT4* was also observed following a 20-h IFN-γ treatment. In addition, we observed an increase in the levels of both C/EBPβ and C/EBPδ following an IFN-γ treatment. A notable decrease in *aP2/422* was observed after a 12-h treatment with IFN-γ. The expression of glycerol phosphate dehydrogenase (*GPD*), a gene whose expression is elevated in adipocytes, was substantially decreased following a 20-h treatment with IFN-γ. Following a 24-h IFN-γ treatment, there was also a slight decline in *adipsin* mRNA. The hybridization of β-actin is shown to represent the even loading of the samples.

Because the IFN-γ-induced loss of PPARγ mRNA was relatively rapid, we predicted that the decay of the PPARγ mRNA

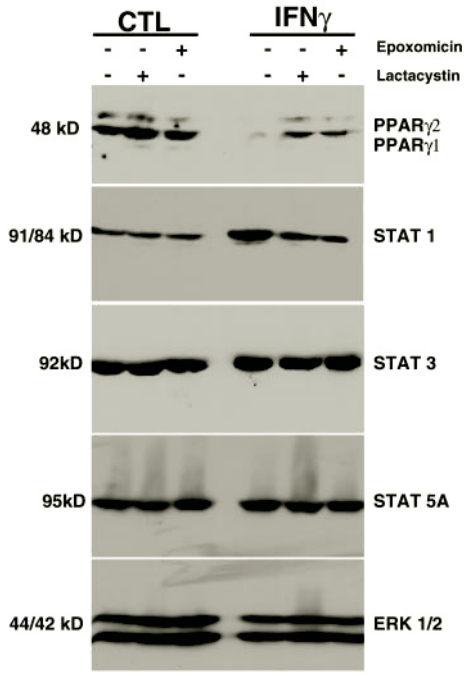

FIG. 7. **The IFN-γ-induced decrease of PPARγ is partially inhibited in the presence of proteasome inhibitors.** Whole cell extracts were prepared from fully differentiated 3T3-L1 adipocytes following a 6-h treatment of either 100 nM epoxomicin or 5 μM lactacystin in the presence or absence of IFN-γ (100 units/ml). One hundred μg of each extract was separated by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blot analysis. Samples were processed and results were visualized as described in the legend to Fig. 1. This is a representative experiment independently performed three times. *CTL*, control.

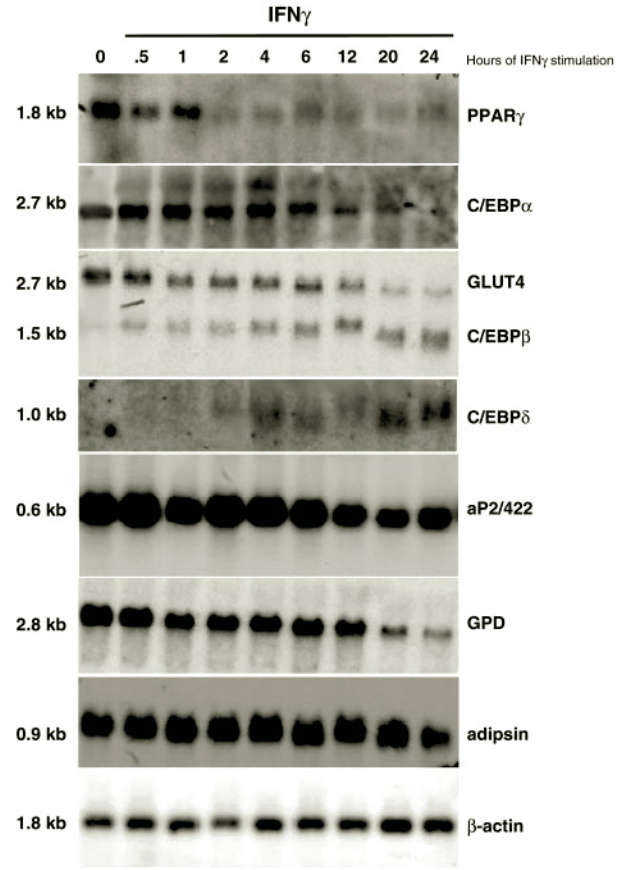

FIG. 8. **IFN-γ treatment results in a rapid loss of PPARγ mRNA and a decrease in expression of other adipocyte markers.** Total RNA was isolated from fully differentiated 3T3-L1 adipocytes following treatment with IFN-γ as indicated at the *top* of the figure. Twenty μg of total RNA was electrophoresed, transferred to nylon, and subjected to Northern blot analysis. This is a representative experiment independently performed two times. *GPD*, glycerol phosphate dehydrogenase.

would be brief compared with C/EBPα. Therefore, we investigated the turnover of these two transcription factor mRNAs. Total RNA was isolated from cells at various times after treatment with actinomycin D. As shown in Fig. 9*A*, the PPARγ mRNA decayed rapidly compared with the C/EBPα mRNA. We estimated the half-life of the PPARγ mRNA to be less than 3 h. We also examined the decay of PPARγ in the presence of IFN-γ to determine whether this growth factor had any effect on the stability of the PPARγ mRNA. We found that the decay of PPARγ mRNA was not altered in the presence of IFN-γ as indicated in Fig. 9*B*. These results strongly suggested that IFN-γ would have an effect on the transcription of PPARγ.

To determine whether the IFN-γ-induced changes in PPARγ and C/EBPα mRNA accumulation shown in Fig. 8 were attributable to the effects on synthesis, we measured the transcription rates of these genes in nuclei isolated from control and IFN-γ-treated adipocytes. Fully differentiated 3T3-L1 adipocytes were exposed to cycloheximide (±IFN-γ) for 1 h. As shown in Fig. 10, a substantial suppression of both PPARγ and C/EBPα was observed following IFN-γ treatment, indicating that the effect of IFN-γ on the transcription of these genes was independent of new protein synthesis. IFN-γ had no effect on β-actin transcription (data not shown).

IFN-γ is known to have effects on both lipolysis and lipogenesis, so we investigated the effect of this growth factor on basal and insulin-sensitive glucose uptake. As shown in Table I, serum-deprived 3T3-L1 adipocytes had a 6.7-fold increase in glucose uptake following a 10-min treatment of 100 nM insulin. After a 24-h treatment of IFN-γ, cultured adipocytes were still responsive to insulin (6.13-fold increase). However, following a 48-h treatment of IFN-γ, when both PPARγ and C/EBPα were substantially decreased (Fig. 1), there was a discernible decrease in insulin-stimulated glucose uptake (4.42-fold increase). Exposure to IFN-γ for 72 and 96 h had no effect on basal glucose uptake but resulted in a substantial decrease in insulin-sensitive glucose uptake. Following a 96-h IFN-γ exposure, there was only a 2.2-fold increase following insulin treatment. IFN-γ treatment for more than 96 h did not result in a further decline of insulin-sensitive glucose uptake (data not shown). The IFN-γ-induced effects on insulin sensitivity do not appear to be a result of any significant lipid loss as there were no distinguishable differences in Oil Red O staining from control and IFN-γ-treated (96 h) adipocytes (data not shown).

DISCUSSION

IFN-γ affected the expression of many adipocyte transcription factors, including PPARγ2 and -γ1, C/EBPα, C/EBPβ, C/EBPδ, SREBP-1, STAT 1, and STAT 3. However, the most profound effect of IFN-γ was on PPARγ expression. These studies have also revealed that both the PPARγ mRNA and protein are labile compared with other adipocyte transcription factors. IFN-γ treatment of adipocytes leads to a decrease in PPARγ that is the result of the inhibition of transcription coupled with an increase in the degradation of PPARγ2 and -γ1. Interestingly, recent studies have revealed that thiazolidinedione treatment of the 3T3-F442A adipocytes results in a reduction of PPARγ protein that is distinct from mRNA regulation (18). In that study, the data indicated that the TZD treatment of adipocytes resulted in the ubiquitination of PPARγ and subsequent degradation that was dependent on the proteasome complex (18). These results are comparable with the effects we observed with IFN-γ. In our studies, two distinct

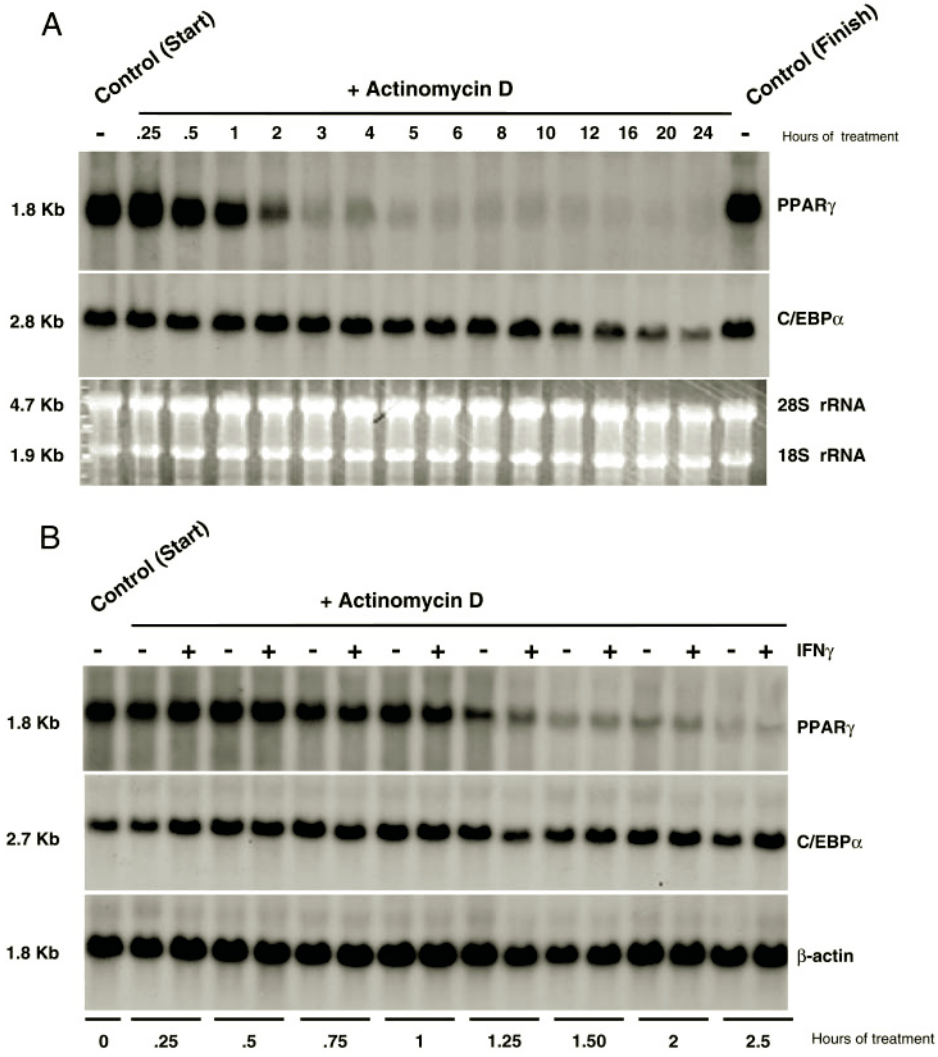

FIG. 9. **PPARγ mRNA is more labile than C/EBPα mRNA in adipocytes, and the decay of these mRNAs is not affected by IFN-γ.** *A*, total RNA was isolated from fully differentiated 3T3-L1 adipocytes following treatment with 5 μg/ml actinomycin D for the various periods of time indicated at the *top* of the figure. Control samples (−actinomycin D) were isolated at the start and finish of the experiment. *B*, total RNA was isolated from fully differentiated 3T3-L1 adipocytes following treatment with 5 μg/ml actinomycin D for the various periods of time indicated at the *top* of the figure in the absence or presence of IFN-γ. In each experiment, 20 μg of total RNA was electrophoresed, transferred to nylon, and subjected to Northern blot analysis for C/EBPα and PPARγ. This is a representative experiment independently performed two times.

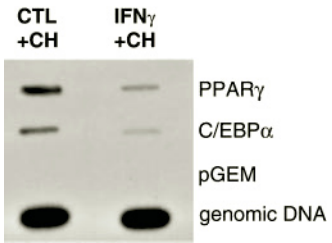

FIG. 10. **IFN-γ treatment results in a suppression of PPARγ and C/EBPα transcription in adipocytes in a manner that is independent of new protein synthesis.** Nuclei were isolated from fully differentiated adipocytes that were exposed to 5 mM cycloheximide in the presence or absence of IFN-γ for 1 h. Nuclei were subjected to run-on analysis, and the autoradiogram displayed isa representative of an experiment performed twice with independent preparations of nuclei. *CTL*, control.

TABLE I
The effect of IFN-γ on insulin-sensitive glucose transport

	Basal	Insulin-stimulated	-Fold increase
	pmol/min/mg protein		
Control	67 ± 12	449 ± 38	6.70
24-h IFN-γ	64 ± 4	392 ± 40	6.13
48-h IFN-γ	66 ± 10	292 ± 28	4.42
72-h IFN-γ	56 ± 6	212 ± 20	3.80
96-h IFN-γ	53 ± 8	117 ± 13	2.20

proteasome inhibitors affected PPARγ2 and -γ1 protein levels in the presence of IFN-γ but had little effect in adipocytes lacking cytokine stimulation. In summary, both TZD and IFN-γ treatments of adipocytes appear to target PPARγ to the proteasome for degradation. Moreover, our observations on the lability of PPARγ proteins suggest that PPARγ turnover is an important event.

The inhibition of proteasome activity in the presence of IFN-γ did not restore the PPARγ proteins to normal cellular levels, and we observed a potent effect of IFN-γ on PPARγ transcription and mRNA accumulation. These results have led us to hypothesize that IFN-γ-induced STAT 1 activation in fat cells may be responsible for the transcriptional suppression of PPARγ. Cross-talk between STATs and PPARs has been demonstrated in liver cells (22). We are currently initiating studies to identify the IFN-γ-sensitive element in the PPARγ promoters and determine whether STAT 1 is directly involved in the transcriptional suppression of PPARγ. Although STATs are generally thought to be transcriptional activators, there is evidence that this family of transcription factors can also act as repressors of transcription (23). These studies have led us to hypothesize that IFN-γ-induced STAT 1 dimers directly bind to the PPARγ promoters and result in an inhibition of transcription.

The effects of IFN-γ on PPARγ degradation are less expected. Numerous studies have shown that serine phosphorylation of PPARγ on Ser^{112} by mitogen-activated protein kinases (ERK1/ERK2 and stress-activated protein kinase/c-Jun NH_2-terminal

kinase) results in a strong suppression of PPARγ activity (24–27), which in part appears to involve ligand binding (28). Our previous studies in the 3T3-L1 adipocytes have demonstrated that IFN-γ resulted in both STAT 1 and STAT 3 tyrosine phosphorylation and nuclear translocation (15). However, unlike other cytokines, IFN-γ did not result in the activation of ERK1/ERK2 in adipocytes. Therefore, it does not appear that ERK1/ERK2-induced serine phosphorylation of PPARγ could be involved in the effects of IFN-γ that we observed on PPARγ degradation. Our results are supported by the findings of Spiegelman and co-workers (18), which indicate that the phosphorylation of PPARγ on Ser^{112} is not required for its down-regulation. However, we have not examined the role of serine phosphorylation in the IFN-γ-induced PPARγ degradation or the effect of IFN-γ on the activation of c-Jun NH_2-terminal kinase in adipocytes.

Although the mechanism by which IFN-γ directs PPARγ to the proteasome for degradation is not known, it is clear that the turnover of PPARγ is further increased when both IFN-γ and a PPARγ ligand are present. Perhaps IFN-γ could either modulate the phosphorylation state of PPARγ or have an effect on the synthesis of an endogenous PPARγ ligand. Alternatively, IFN-γ-induced PPARγ degradation could occur via a pathway that is independent of ligand-induced degradation. It is interesting to note that the analysis of PPARγ mutants by the Spiegelman laboratory demonstrated that the TZD-induced PPARγ decay was not strictly dependent on its transcriptional activity but was dependent upon the ligand-gated activation function (AF-2) domain. In these studies, ligand binding and the activation of the AF-2 domain not only increased the transcriptional function of PPARγ but also induced ubiquitination and subsequent proteasomal degradation.

Unlike TZDs, which are insulin sensitizers, IFN-γ treatment of adipocytes resulted in a condition of insulin resistance, as measured by insulin-sensitive glucose uptake and a decrease in the expression of adipocyte genes, such as *GLUT4*, *aP2/422*, *GPD*, and *adipsin*. PPARγ has been implicated in the regulation of systemic insulin sensitivity, and some PPARγ mutations are associated with severe insulin resistance and diabetes mellitus (29). In our studies, the most profound effect of IFN-γ was on PPARγ expression, which was significantly decreased after only 6 h. Interestingly, we did not observe any substantial effects on insulin-sensitive glucose uptake even after a 24-h treatment of IFN-γ despite the dramatic loss of PPARγ expression. Following a 48-h treatment, we did observe a substantial inhibition of insulin-sensitive glucose uptake. At this time, there was also a marked effect on C/EBPα expression. These studies suggest that the loss of PPARγ may be insufficient to confer insulin resistance in 3T3-L1 adipocytes. However, the low levels of PPARγ observed after a 24- and 48-h IFN-γ treatment may be sufficient levels of PPARγ expression to account for the insulin responsiveness of these cells. Alternatively, the primary role of PPARγ may be to regulate the expression of other transcription factors, such as C/EBPα. Nonetheless, the increase in PPARγ turnover and the inhibition of PPARγ synthesis induced by IFN-γ are prominent because of the relatively rapid decay of both the PPARγ mRNA and the protein. Because the regulation of PPARγ is the first observed effect of IFN-γ on adipocyte transcription factor expression, this event is likely very important in the development of IFN-γ-induced insulin resistance. IFN-γ treatment also results in a decrease of *GLUT4*, *aP2*, *GPD*, and *adipsin* expression in adipocytes. However, there is no notable difference in the morphology of the cells, and there is no observable difference in Oil Red O staining from untreated 3T3-L1 fully differentiated adipocytes and those that have been treated for 96 h with IFN-γ (data not shown). In conclusion, the tightly controlled regulation of PPARγ synthesis and degradation that we observed in the presence of IFN-γ suggests that the cellular levels of PPARγ are a meaningful effector of gene expression.

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